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Pectin and Alginate Extraction to Treat Liquid CAFO Manure

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PECTIN AND ALGINATE EXTRACTION TO TREAT LIQUID CAFO MANURE

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HONORS PROJECT

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at Bowling Green State University in partial fulfillment of the

requirements for graduation with

UNIVERSITY HONORS MAY 1 2021

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Abstract

For this project, various extraction methods were used to extract pectin from *Pastinaca Sativa* and alginate from *Macrocystis*. These extractions were then dried and used in treating 250mL of manure along with a $CaCl_2$ or $FeCl_3$ coagulant. It was found that $CaCl_2$ was not as effective as $FeCl_3$ in coagulating manure. But the results obtained suggest that pectin and alginate obtained with a simpler extraction method is just as effective as the highly purified and refined pectin and alginate produced for the food industry, in the treatment of CAFO manure. The liquid portion of the manure treatments were tested for the dissolved nutrients of nitrate, ammonia, and phosphorus. Results suggest that, and the polysaccharides obtained by the simpler extraction method are as effective as highly purified polysaccharides. More testing needs to be done to determine effectiveness for binding ammonia. Next steps should include testing the length of time needed for sonication that would yield the highest levels of pectin and alginate. Another next step would be testing the amount of dissolved nutrients in the parsnip and seaweed before extraction.

Introduction

Since the middle of the 1990s, harmful algal blooms (HABs) have become an increasingly severe problem in large bodies of water, including Lake Erie. HABs are overgrowths of cyanobacteria in water that sometimes produce harmful toxins that can have severe impacts on aquatic ecosystems and on human health[1]. HABs are partially being caused by the phosphate, nitrate, and ammonia in fertilizers that become runoff after storms and get into these bodies of water. There are other causes to HABs, but this project focuses solely on the fertilizers being used. The toxins produced by cyanobacteria can kill fish and other aquatic animals, as well as make the water toxic for humans and animals.

In the farming industry, it is common that manure is used as a fertilizer as it can provide nutrients to the soil. The manure used in this experiment is from large Concentrated Animal Feeding Operations (CAFO), specifically dairy cows. The manure from these farms is mostly water, so the main goal is to first extract the solid material and nutrients out of the material. In dairy systems, the mechanical solid separators of manure from liquid are only 20% effective[2]. The liquid manure can be put on fields as a fertilizer by itself but easily becomes runoff because of the high-water concentration and solubility of the nutrients. Fresh manure is expensive to transport, so ideally the manure would be used on the same farm, or any neighboring farm. The overall goal of this research is to find the best possible way to reduce levels of nutrients that are washed out of farm fields into the watershed and ultimately into these lakes and streams. This is being done by testing ways in which phosphate, nitrate, and ammonia stay in the soil/manure instead of becoming runoff and the main way this has been done is to use coagulants and photopolymers or polymers. The polymers help bind the solid manure together to extract it from the liquid. Aluminum sulfate, lime, ferric sulfate, ferric chloride, and aluminum chloride all have been used in the past as coagulants for the manure and to reduce phosphate levels[2].

The two polymers that were used in this project were pectin from *Pastinaca Sativa* and alginate from *Macrocystis*. *Pastinaca Sativa*, or parsnips, are root vegetables closely related to carrots that have a high fiber content. Out of all vegetables and fruits, parsnips have some of the highest pectin content, so it was chosen for this project. Pectin is commonly used in the solidification of jams and jellies. *Macrocystis* is a genus of kelp that used to be harvested annually in California, about 100-170 thousand wet tons a year[3]. *Macrocystis* is known to be used for alginate extraction and for feeding abalones[3]. Pectin is a complex polysaccharide that is a common food ingredient that is found in gelling agents[4]. It has three main properties that make it a good gelling agent including being colloidal, being soluble, and gelation. In a commercial setting, pectin is prepared from apple pomace and citric peels by acid extraction and filtration, and then a precipitation with alcohol[5]. Figure 1 shows the structure of pectin.

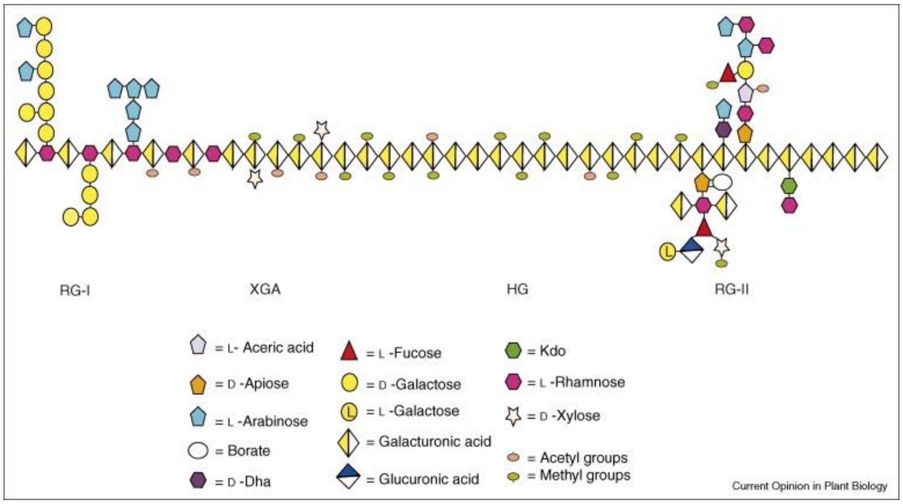

Figure 1. Molecular structure of pectin[6]

Alginate on the other hand is an anionic polysaccharide found in the cell wall of many species of seaweed[7]. One important characteristic of alginate is that it can interact with divalent (Ca^{2+}) and trivalent cations (Fe^{3+})[7]. The purification of alginate is a major roadblock for many studies on translational application[7]. Figure 2 shows the structure of alginate.

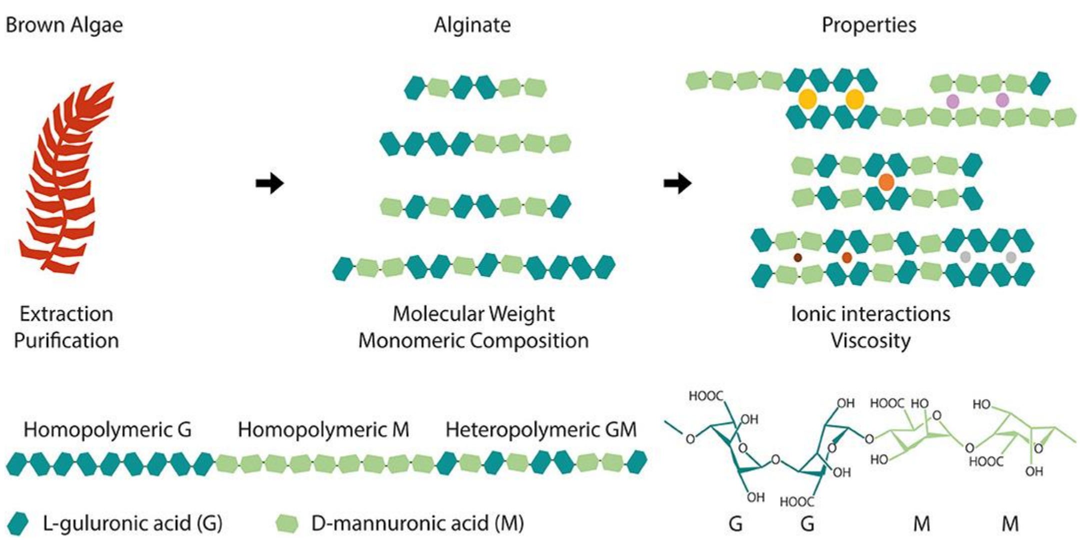

Figure 2. Molecular structure of alginate[8]

Materials and Methods

There were three major parts to the methods of this project: extraction, treatment, and testing.

Extraction

The extraction procedure was adapted from Bui, A et al[9]. First, whole parsnips were sliced with a mandoline and reduced to a pulp with a food blender and addition of water. The dried seaweed was ground using a mortar and pestle into small flakes. Both materials were then weighed and suspended in water in 50mL centrifuge tubes. Two different methods were used to lyse the cells, the first being a bath sonicator. These centrifuge tubes were sonicated in an Ultrasonic Bath (Fisher Scientific) for an hour to break up the cells. After completing a few extractions, there was no noticeable change in the viscosity of the sample so a probe sonifier (250 Sonifier Analog Cell Disruptor, Branson Ultrasonics Corporation) was used on the rest of the project to lyse the cells. The samples were first frozen with liquid nitrogen until solid and then thawed. There were two sizes of samples, beakers and 50mL centrifuge tubes. 500mL Beakers of sample were sonified for a total 30 minutes with a 1.5-minute break every minute. 50mL Centrifuge tubes of sample were sonified for a minute with a 1.5-minute break every 10 seconds. The sonification was not done all at once as the tip of the Sonifier can reach up to 60-70°C if on for long periods of time and polysaccharides can start to break down at those temperatures. Sonicated/sonified samples were spun down in a centrifuge (Sorvall ST 16R, Thermo Scientific, #41630611) at 3000rpms for 2 minutes and the supernatant was decanted into new 50mL centrifuge tubes in 10-12mL portions. 4 volumes of 200 proof ethanol (Ethyl alcohol denatured, Sigma Aldrich, #SHBM4878) were added to precipitate the polysaccharide. After mixing the tubes, the samples were centrifuged, and the supernatant was discarded. Extracted pectin or alginate and other polysaccharides were dried and weighed.

Treatment

The dried extracted samples of pectin and alginate were ground to a powder using mortar and pestle. These samples were suspended in water at 1g/100mL or 1g dry extract was used. The manure treatment was done two separate ways depending on the state of the polymer, whether it was solid or liquid. For the liquid polymer, first 100mL of coagulant ($CaCl_2$ or $FeCl_3$) was added to 250mL of manure. This was then mixed for 20 minutes in an automated mixer (Lovibond ET 750 floc tester). The polymer suspension was then added and mixed for additional 20 minutes. For the solid polymer, 1g of polymer was added to the 250mL of manure and mixed for 20 minutes. Then 100mL of coagulant was added and mixed for additional 20 minutes. All the manure treatments were left to sit overnight to allow for complete separation. The next day, the treatments were separated into solids and liquids by vacuum filtration. The solids were dried and massed and the liquid portions were analyzed for pH, turbidity, color, volume, and analyzed for dissolved phosphate, nitrate, and ammonia.

Testing

Various instruments were used to analyze the liquid portion of the manure. A pH meter (HACH HQ11D) was used to measure pH of the liquid and a turbidity meter (HACH 2100Q) was used to measure the turbidity of the liquid. The color was measured against a standard color scale ranging from a light gold to cedar brown. The color scale was developed in the Midden research group to establish a standard for uniform evaluation of the effectiveness of manure treatment. The dissolved phosphate, nitrate and ammonia was analyzed using a Seal Analytical AQ2 Discrete Chemical Analyzer (#090796).

Results

The results of the extractions can be seen in Tables 1 and 2. The results for the manure treatments can be seen in Table 3. And the results for determination of phosphate, nitrate, and ammonia in the filtrate can be found in Table 3 and Figures 3, 4, and 5.

In Table 1, the S is for the seaweed samples and the P is for the parsnip. Samples S1-P2 were all extracted with bath sonification, S4-P200 were extracted with the probe sonifier, and the parsnip sample had no sonification before extraction. A peer reviewed paper that was found to estimate percent yields of pectin reported results based on dry mass of parsnip so all of the parsnip samples had to be adjusted for water weight[10]. To adjust for water content, 18.83g of the blended parsnip was put into a petri dish and dried in an oven at ~60° C. After complete dehydration, the weight of the parsnip was measured to be 1.579g which would make the parsnip samples 91.6% water. The percent yield was calculated by dividing the adjusted extracted amount by the theoretical yield. The theoretical yield was determined using data from the previously published study of pectin extraction.

Table 1: Pectin and Alginate extraction results

Sample	Dry mass (g)	Adjusted for wc (91.6%)	Extracted (g)	Adjusted for wc (91.6%)	Theoretical (g)	Percent yield (%)
S1	14.10		0.42		2.82	14.90
P1	50.00	4.20	0.34	0.03	0.31	9.25
S2	16.41		0.83		3.28	25.32
P2	83.65	7.03	0.74	0.06	0.51	12.13
S4	15.27		0.81		3.05	26.36
P4	85.61	7.19	1.67	0.14	0.53	26.67
P100	50.00	4.20	0.66	0.06	0.31	17.93
P50N	25.00	2.10	0.29	0.02	0.15	15.87
P50S	25.00	2.10	0.33	0.03	0.15	17.84
P200	100.00	8.40	1.13	0.09	0.61	15.46
Parsnip	102.5	8.61	0.6	0.05	0.63	8.01

In Table 2, the Parsnip again and the Seaweed again are both samples that were reextracted. As there was no viscosity change in samples S1-P2 in Table 1, the discarded parsnip and seaweed were saved and resonicated to see if any more pectin or alginate could be extracted if the material was better sonicated. With samples 1:1-1:7, the ratio was changed between the amount of parsnip and the amount of water added. The question was whether larger amounts of water would allow for more pectin to be extracted.

Table 2: Pectin and alginate results for varied outcomes

Sample	Dry weight (g)	Adjusted for WC (91.6%)	Water (mL)	Extracted (g)	Adjusted for WC (91.6%)	Theoretical (g)	Percent Yield (%)
Parsnip again	~200	16.8	200	1.70	0.1428	1.181	12.1
Seaweed again	~200	---	200	1.27	---	----	-----
1:1	25.43	2.14	25	0.21	0.018	0.16	11.5
1:2	14.941	1.26	30	0.11	0.009	0.092	9.82
1:3	10.151	0.85	30	0.09	0.008	0.062	12.3
1:4	10.072	0.85	40	0.05	0.005	0.062	7.30
1:5	7.201	0.60	35	0.05	0.004	0.044	9.30
1:6	7.075	0.59	42	0.05	0.004	0.043	9.2
1:7	6.073	0.51	42	0.04	0.004	0.037	9.87

Table 3 shows the results from the manure treatments. Samples 3-6 were treatments done with refined pectin (Pectin from citrus peel, Sigma Aldrich, #SLBS8828) or refined alginate (Alginic acid sodium salt from brown algae, Sigma Aldrich, #SLBT1732) with 0.1M $FeCl_3$ as the coagulant. Samples 7-11 were treatments done with extracted pectin or extracted alginate with 1M $CaCl_2$ as the coagulant. Sample 11 was done with highly purified alginate (KIMICA, sodium alginate, #CHILE7K17301) as a treatment that has been previously studied and known to be effective. Samples 7-11 were the first treatments done and as the masses of the dry cakes were very low, samples 3-6 were done to confirm that the treatments were successful before continuing with any more extracted treatments. Samples 12-16 were a second round of extracted pectin and alginate manure treatments done with 0.1M $FeCl_3$. The solid/liquid column refers to the polymer being added as either a solid or liquid. Color is the color of the filtrate. Volume is the amount of filtrate. And the mass of the cake is the weight of the manure after filtration and drying.

Table 3: Manure Treatment Results

Sample #	Sample name	Bucket	Coagulant	Amount (mL)	Polymer	Amount	Solid/liquid	Color	pH	Turbidity (NTU)	Volume (mL)	Mass of dry cake (g)
3	FAS	21	0.1M FeCl3	100	alginate	1.0g	solid	teak	8.5	410	144	11.017
4	FPS	21	0.1M FeCl3	100	pectin	1.02g	solid	teak	6.8	782	170	10.767
5	FAL	21	0.1M FeCl3	100	alginate	1.01g/100mL	liquid	rattan	7.8	85.8	270	10.864
6	FPL	21	0.1M FeCl3	100	pectin	1.0g/100mL	liquid	sand	7.2	318	380	10.37
7	1CPL	21	CaCl2	20	pectin	126mL	liquid	cedar	8.1		341	1.167
8	1CPS	21	CaCl2	20	pectin	0.97g	solid	cedar	7.8		174	1.633
9	1CAL	21	CaCl2	20	alginate	40mL	liquid	cedar	8.1		166	2.123
10	1CAS	21	CaCl2	20	alginate	1.1g and 40mL	solid/liquid	cedar	8.1		155	2.874
11	factory	21	CaCl2	20	alginate	1.03	solid	cedar	8.1		151	2.393
12	2PLB	21	0.1M FeCl3	100	liquid pectin	0.68g/100ml	liquid	gold	6.3	64.6	390	10.858
13	2PSB	21	0.1M FeCl3	100	solid pectin	0.71g	solid	gold	6.2	23.9	350	5.413
14	2PLN	21	0.1M FeCl3	100	liquid pectin	0.91g/100ml	liquid	gold	6.2	38.8	410	9.641
15	2PSN	21	0.1M FeCl3	100	solid pectin	1.01g	solid	gold	6.2	48.1	314	11.434
16	2ASN	21	0.1M FeCl3	100	solid alginate	0.81g	solid	rattan	6.2	43.9	326	5.275

Figures 3-5 show the concentration of the various nutrients being tested for in the liquid portions of each sample. Samples 1 and 2 are manure samples without any treatment, samples 3-16 correlate to the same sample numbers seen in Table 3.

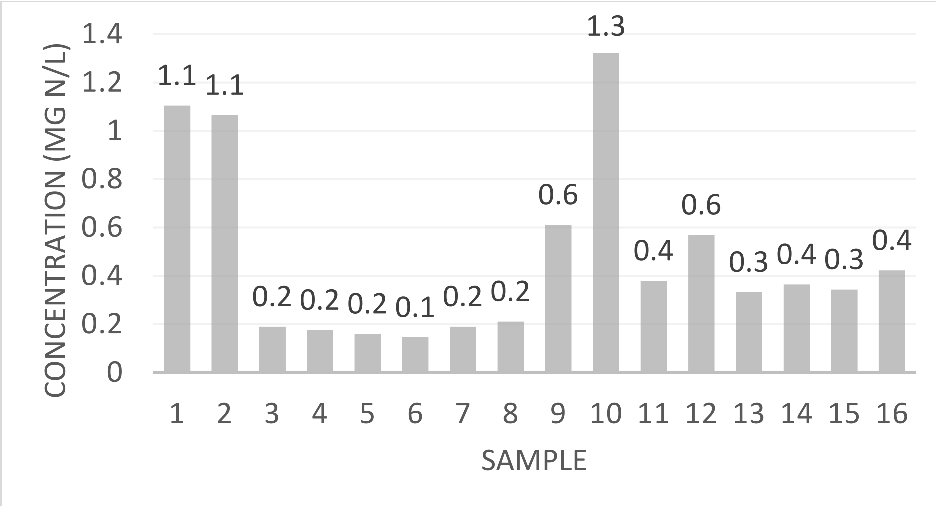

Figure 3: NO_3/NO_2 concentration in liquid portion of manure treatments

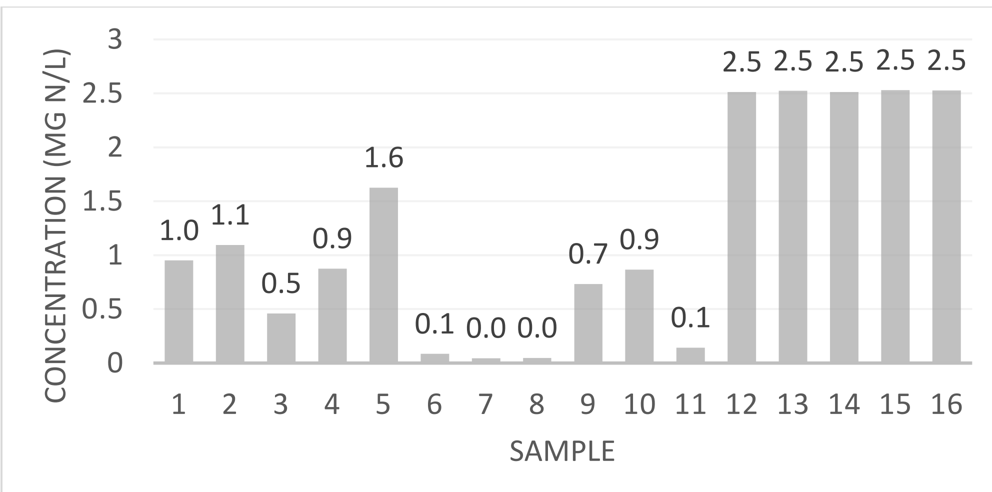

Figure 4: Ammonia concentration in liquid portion of manure treatments

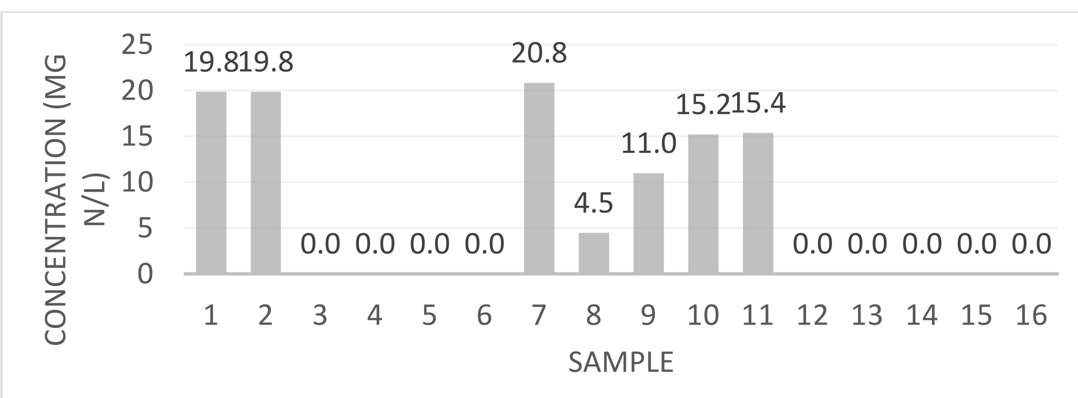

Figure 5: Phosphate concentration in liquid portion of manure treatments

Discussion

Extractions

The results in Table 1 suggest that bath sonification is not as effective at breaking up the cells as the Branson probe sonifier. This is shown in the percent yield difference of 9.25% of P1 which was sonicated in the bath compared to the 17.93% of P100. Moving forward, the Branson probe sonifier should be used to lyse the cells. Comparing any of the parsnip sonification samples to the parsnip sample without sonification, it can be seen that sonicating the samples results in a higher percent yield than extracting from blended parsnip. This difference would likely be even larger comparing extracted material from the sonicated material to a fresh parsnip. The lower percentage yields suggest that longer sonification may increase the amount of pectin and alginate extracted.

The results in Table 2 suggest that more material that was originally treated in the bath sonificator could be extracted after resonification. As there was no viscosity change in the material after bath sonification, the discarded parsnip and seaweed were saved and resonicated and more polysaccharides were extracted. These results also suggest that changing the parsnip to water ratio has no effect on the amount of pectin extracted. As the larger ratios had similar percent yields to the smaller ratios, it is just as effective to have 25g of parsnip suspended in 25mL of added water as 6g of parsnip suspended in 40mL of water.

Treatment

The results for samples 7-11 in Table 3 show that 20mL of $CaCl_2$ is not effective in coagulating 250mL of manure because of the low dry cake mass and the color of the liquid. Ideally, the liquid after filtration should be a gold or sand color, but because they were all a cedar color indicates that not all of the manure was coagulated. Using $CaCl_2$ as a coagulant could still be effective, but not in the amount that it was used because also having the factory alginate treatment sample not work is an indicator that the issue in coagulation was not the polymers. By looking at samples 3-6, it is shown that 0.1M of $FeCl_3$ is effective in coagulating manure because of the 10-11g of dry cake mass. But the larger amount of $FeCl_3$ used may also account for the different results. The slightly darker color of the liquid could indicate that not all the manure was coagulated but it is significantly more effective than the treatments with the 20mL $CaCl_2$. The very high turbidity also indicates a high level of particulate organic matter still in the liquid. Finally, looking at samples 12-16, it can be proposed that the less refined pectin and alginate are just as effective, if not more effective, in coagulating manure as factory refined highly purified pectin. Using the same coagulant as samples 3-6, $FeCl_3$, the dry cake masses of samples 12-16 were similar or even higher than the factory refined treatments. Not all of these samples can be completely comparable just by looking at the weights as only sample 15 out of 12-16 used a full gram of polymer as there was not enough extracted material for four 1g polymer treatments. Even with this, though, many samples with lower polymer amounts coagulated comparable manure amounts to the factory refined samples with ideal amounts of polymer. The color and low turbidity of these last four samples also suggests a better coagulation of manure.

Testing

Lastly, looking at Figures 3-5 can lead to a few propositions. Again, samples 1 and 2 are the liquid manure without any treatment and effective treatments would result in lower nutrient levels in the filtrate. For the nitrate levels, all of the samples, except sample 10, had lower concentrations of nitrate in the filtrate than the starting manure suggesting that most of the nitrate was captured in the treatments. But with the highly purified polymers the filtrate had lower levels of ammonia than the starting manure but the second self-extracted pectin and alginate samples had double the concentration of ammonia of the starting manure. There are multiple reasons that may explain this, one being the possible ammonia content of parsnip and seaweed. While this might not be the case, testing for this is warranted. Lastly, the phosphate levels in filtrate produced with both the highly purified and less purified polysaccharides was essentially zero, leading to the conclusion that these treatments were successful in capturing all of the phosphate in the manure.

Conclusions

These results suggest that less refined pectin can be as effective as highly refined pectin for manure treatment. There is not a high confidence in this as there were only five treatments done with the extractions but there is reasonable confidence in the effectiveness for binding phosphate and nitrate. But testing should be continued as there was variation in ammonia concentration. The Branson Probe Sonifier is more effective at breaking up cells than the bath sonicator. Changing the ratio of parsnip to water did not have a significant effect on the amount of pectin extracted as previously thought. This extraction was more effective for pectin from parsnips than alginate from *Macrocystis* as it was harder for the sonifier to break up the large flakes of seaweed. This method may be more effective for the alginate if the dried seaweed were to be ground into a powder using a grinding mill. If this is still not very effective then other methods should be tested for *Macrocystis*. A rough estimate indicates that this pectin extraction method substantially reduces cost from \$0.39/g to \$0.02/g but this analysis needs to be revised to include cost of labor.

There are several suggestions next steps. Testing the pectin/alginate extractions for effectiveness in manure treatment should be conducted with varying amounts of $FeCl_3$ and concentrations of the pectin/alginate. This should include retesting the highly refined pectin with larger quantities of $CaCl_2$ to determine if that yields a better result. The sonication time should be varied to find maximum the time of highest yield. As the machine used for sonification was switched halfway through the semester, there was not enough time to test for how long the material should be sonicated. Lastly, the starting amounts of phosphate, nitrate, and ammonia in the parsnip and seaweed should be determined as some of the treatments had higher levels of these nutrients than the untreated manure.

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